Circulation Type Blood Vessel Simulator Made by Microfabrication

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論文内容要旨

Surgical simulators are used in practice and rehearsal for intravascular neurosurgery, and for development of new medical instruments such as catheters. Tailor made three-dimensional (3D) elastic membranous blood vessel models have been developed with 3D wax models fabricated by ink jet rapid prototyping. Then, transparent surgical simulators are developed by connecting the elastic membranous models. Blood vessels in a conventional surgical simulator are larger than 500 μm in diameter. Smaller blood vessel models are needed to simulate a more realistic vessel environment; however, these are very difficult to fabricate using ink jet rapid prototyping because of the brittleness of the wax. Diseases such as arteria basilaris exist in blood vessels that are narrower than ϕ500 μm. Previous surgical simulators are not suitable for rehearsal and training for such diseases. This dissertation aims to fabricate multiscale transparent arteriole and capillary vessel models that enable easy simulation of blood circulation. For this purpose, microchannels should de fabricated with circular cross sections of ϕ10–500 μm. Many methods such as machining, stereolithography, ink jet rapid prototyping, and photolithography have been proposed for fabricating microchannels. Machining is suitable for a straight channel up to around 10 μm in diameter, but not for complicated capillary vessel networks. Stereolithography is applicable for fabricating a mold; however, it is difficult to dissolve it to create a hollow structure. Ink jet rapid prototyping is beneficial for thicker channels such as aortas, but not applicable for capillary vessel models. Photolithography is a fundamental technology for fabricating microchannels, and a high resolution around 1 μm is easily attained. Therefore, photolithography has been chosen for fabricating arteriole and capillary vessel models. In general, fabricating microchannels with a circular cross section is quite complex.

Microchannels were fabricated using semicircular cross section photoresist patterns and light curable resin, but the cross sections of the fabricated channels were semicircular. These processes are not suitable for fabricating fine blood vessel models. Therefore, a new fabrication process for multiscale transparent arteriole and capillary vessel models were proposed in this dissertation. The fabricated arteriole and capillary vessel models are connected with conventional blood vessel models to realize a circulation simulator. For example, circulation

models will allow simulation of animal testing and drug delivery systems, by using microvessels. In this dissertation, fabrication methods and prototyping results for the circulation models are reported.

This dissertation reported six research topics that can be categorized into following three areas; (i) Various type of arteriole and capillary vessel models for blood vessel simulator (Chapter 2, 3, 4), (ii) Connecting methods for realizing circulation type blood vessel simulator (Chapter 5) and (iii) Micromachining for surface of biocompatible polymer (PDMS) (Chapter 6).

In chapter 1, I introduced the background of conventional surgical simulators, and explained the needs of arteriole and capillary vessel models as surgical simulator for high precision simulating. For fabricating these models, photolithography process was selected in many maicrofabrications.

In chapter 2, multi scale fabrication method of ϕ10 - 500 μm arteriole and capillary vessel models was proposed and demonstrated. It is necessary to choose an appropriate exposure method. From the experimental results, it was confirmed proposed method is useful for making arteriole and capillary vessel models. These models were fabricated by photolithography and plasma bonding with patterned PDMS substrates. In fabricating capillary vessel models, when two PDMS substrates assembled, alignment is very difficult and patterns are out of alignment. To solve this problem, new patterns are needed for PDMS substrates assembly. And, capillary vessel pattern and alignment pattern for assembly were fabricated by multi stage exposure photolithography. And, the alignment accuracy of the capillary vessel models was evaluated. A model having no alignment pattern had an alignment error of 2.5 μm, whereas, a model with alignment patterns had an alignment error of 0.6 μm. The circularity of the capillary vessel model having no alignment pattern was 70% and for that with alignment patterns was 84%. Thus, alignment patterns reduced the alignment error from 2.5 to 0.6 μm and improved the circularity from 70 to 84%.

And, the circularity of fabricated microchannels was calculated. Fabricated microchannels were 10 μm in diameter capillary vessel model, 50 μm and 500 μm in diameter arteriole models. The circularity of microchannel is calculated by the shortest axis divided by the longest axis. Using this expression, circularities of ϕ10, 50, 500 μm microchannels were calculated. The circularity of ϕ10 μm microchannel is 84.0%, that of ϕ50 μm microchannel is 61.5%, and that of ϕ500 μm microchannel is 82.3%. All cross-sections of microchannels were circular or elliptical. In application, HUVECs seeding experiments were demonstrated in fabricated ϕ10, 30, 500 μm microchannels. And, seeded HUVECs were adhered in inner all diameter microchannels.

In chapter 3, new fabrication method of ϕ100 - 500 μm arteriole membrane models for surgical simulator was proposed. These blood vessel models are fabricated for connection to the previous surgical simulators. These membrane models will make surgical simulator enable to rehearse and train treatments for diseases at ϕ100 - 500 μm blood vessels, and enable to make surgical simulator with high precision. Fabrication method of arteriole membranous model and evaluation of the molding material (WAX and PVA mixture material) were reported. Arteriole membrane models were made by using grayscale lithography and WAX and PVA mixture material. It is very important to use WAX and PVA mixture material. And, mechanical and chemical properties of WAX and PVA mixture material were evaluated. In mechanical properties, Young's modulus of mixture material was getting higher by increasing the ratio of WAX. In addition, mixture's extension was getting higher by increasing the ratio of PVA in it. In chemical properties, DI water and acetone liquid mixture was most effective for melting WAX and

PVA mixture material. Based on the proposed approach, the brittleness of the previous sacrificial model was overcome. And, I succeeded in making the membranous and hollow structural arteriole model. This arteriole membrane model had circular cross section inside the channel, and circularity of this channel was 90%.

In chapter 4, a ϕ100 – 500 μm transparent networked arteriole model was successfully fabricated. Circularities of fabricated microchannels are calculated by dividing the shortest axis by the longest axis. Using this expression, fabricated network model had ellipse shape cross section and circularity was over 50% under 250 μm in diameter. And, fabricated microchannels had no leakage by the flow experiment. To improve the circularity of microchannels, finding a best exposure condition may be able to solve this issue. Therefore, using grayscale lithography and basing on real vessels' branched rule are useful to fabricate ϕ100 – 500 μm transparent networked arteriole models.

In addition, ϕ20 – 100 μm arteriole network model was successfully fabricated. And, reflowed resist flowed from narrow diameter to large diameter. Therefore, comparing theoretical and experimental circularity, experimental circularity was dramatically improved. Then, reflow method is suitable for fabricating ϕ20 – 100 μm arteriole network models.

In chapter 5, circulation type blood vessel models were fabricated and demonstrated by using arteriole network models and artery models. Two connecting methods were tried. One is a direct connecting method, the other is a seamless connecting method.

In directing method, circulation model was successfully made, however, this model could not have a seamless structure at the connected parts. Therefore, the direct connecting method was not suitable for fabricating circulation models.

Then, a circulation model was fabricated using a wax connector for seamless connecting. Seamless connection method had the advantage of easy connection and good alignment accuracy. The fabricated model had a seamless structure, and it was demonstrated by the flow experiments that this model had no leakage between the connected parts and channels. The proposed connection method was suitable for fabricating circulation models. And, fabricated circulation model can be used for blood vessel simulator from the result of pressure test.

In chapter 6, firstly, it was introduced that the importance of cell-adhesion surface for biodegradable scaffold needed to adhere HUVECs and high-precision blood vessel simulator containing HUVECs. The inner asperity surface structure of the scaffold is porous. The pore size of scaffold has a longer effect on the cellular adhesiveness. This asperity surface structure has effects on behavior and morphology of adherent cells and the auxesis of cells. Therefore, observing cell behavior and morphology of cells helps clarify understanding of the asperity surface structure of scaffold that has quality for attaching cells. For this purpose, the inner asperity surface profile of the scaffold on PDMS substrate was recreated by using photolithography. The concave patterns were made on a PDMS substrate by printing. I plan to refine exposure conditions, development time, etc., so that I can fabricate designed patterns more precisely.

It was observed that HUVECs adhered to the concave patterned PDMS substrates. The tested pattern was 8 μm in diameter and 5 μm deep. The concave distribution density of the tested pattern was 1,600 /mm^2 (40,000 in a 25 mm^2 area). This result might support concave patterns on structure didn't have changes in HUVEC adhesion.

Surface profile and data parameters obtained from cell observations should prove useful for quantitative analysis

of surface conditions. Observing cell behavior and morphology is extremely useful in researching and evaluating microfabrication of cell-adhesion surfaces of scaffolds for synthetic vascular prostheses.

In application, HUVECs seeding experiments was demonstrated on the surface of PLCL scaffold. And, pore size and growth of HUVECs (size: 10–60 μm) are required additional investigation.

And, the fabricated circulation model will be used to evaluate drug delivery systems, diacrisis, and medical treatments by ultrasound.

論文審査結果の要旨

我が国の厚生労働省調べによると，日本における死因別死亡数は第一位に癌，第二位に心疾患，第三位に脳血管疾患があり，これら三つの死因が全体の半分以上を占めている。一般的に，癌・心疾患・脳血管疾患を治療する際は，事前にバーチャルリアリティや流体解析を用いた手術シミュレータを利用してリハーサルを行い，術前計画を立てている。最近では，QOL(Quality of Life)の向上を意識した低侵襲治療を行うことが社会的に求められており，特に前述三疾患を対象とした治療を血管経由で行うことが求められている。最新の治療法研究では，ドラックデリバリーシステム(drug delivery systems: DDS)や肝動脈塞栓療法(trancecatheter arterial chemoembolization: TACE, TAE)などの極細径血管（細動脈・毛細血管）を用いた方法が提案されている。しかし，これまでに開発してきた個人情報に基づく血管シミュレータ(Endo Vascular Evaluator: EVE)では，動脈モデルの作製は可能であるが，極細径血管モデルの作製が困難であった。そこで，微細加工技術（フォトリソグラフィプロセス）を用いて細動脈・毛細血管モデルを作製し，DDS や TAE の評価，ひいては動物実験の代替システムを目標とした循環型血管シミュレータを提案している。本論文は，これらの研究成果をまとめたものであり，全編 7 章からなる。

第 1 章は序論であり，本研究の背景，目的および構成を述べている。

第 2 章では，ブロック型細動脈・毛細血管モデルをマルチスケールで作製する方法を提案し，試作している。モデル作製に用いるフォトリソグラフィプロセスは，作製対象とする血管モデル径によって使い分ける必要があり，オーバー露光法，リフロー法，グレイスケールリソグラフィを使い分けている。提案する作製方法により，高精密な細動脈・毛細血管モデルの作製に成功しており，有効かつ非常に重要な成果である。

第 3 章は，ブロック型モデルでは再現不可能である血管の柔らかさを再現するために，膜型細動脈モデルを自作材料で作製した犠牲モデルを利用することで作製する方法を提案している。本モデル作製に適した自作材料を，引張試験による機械的特性評価と溶解試験による化学的特性評価により選定し，チューブ型の極細径血管モデルを作製することに成功している。これらの提案および結果は，血管シミュレータの高精度化に向けた重要な知見である。

第 4 章は，前章までの単純な Y 字分岐構造の血管モデルではなく，実際の血管分岐法則（マレーの法則）に基づいた細動脈モデルを提案，作製方法を示している。このネットワーク型細動脈モデルの作製の提案は，血管シミュレータ EVE への組み込み実現に向けた非常に重要な成果である。

第 5 章は，作製したネットワークモデルと動脈モデル・静脈モデルを繋ぎ目なく滑らかに接続する方法を提案し，循環型血管シミュレータが実現可能であることを示した。また，作製したモデルを用いて圧力負荷実験を行い，実際の血管における液体の漏れ圧力と同じレベルの結果が得られている。これは提案した循環型モデルの実用化に向けた重要な成果である。

第 6 章は，本シミュレータにおいて DDS や TAE 等の評価を行う際に必要となる，細胞や組織の血管モデル表面での接着性についての実験および結果を示している。また，生分解性ポリマー上でも細胞接着実験を行っており，これは DDS や TAE および動物実験の代替システムを目的としたシミュレータの実用化に向けた非常に重要な知見である。

第 7 章は結論である。

以上要するに本論文は，微細加工技術であるフォトリソグラフィプロセスを用いた循環型血管シミュレータの作製方法を提案し，作製および評価を行うことで，その有効性を示したもので，バイオロボティクスおよび医工学の発展に寄与するところが少なくない。

よって，本論文は博士(工学)の学位論文として合格と認める。